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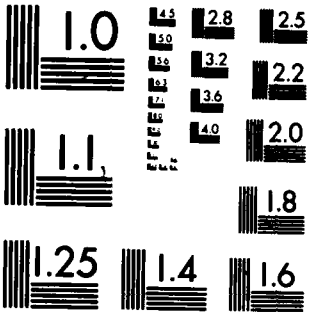

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TNT, 2,4-DNT, metabolism, 4-hydroxylamino-2,6-dinitrotoluene, 4-acetamido-2-nitrotoluene, Azoxytoluene isomers, 2,2', 6,6'-tetranitro-4,4-azoxytoluene 2,2-dinitro-4,4'-azoxytoluene, 4,4'-dinitro-2,2'-azoxytoluene

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Literature review of five compounds, identified as microgial or mammalian metabolites of 2,4,6-Trinitrotoluene or 2,4-Dinitrotoluene, including synthetic and analytical procedures.

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FOREWORD

This work was funded by the U. S. Army Toxic and Hazardous Material agency, under the direction of the U. S. Army Medical Research and Development Command (D. Rosenblatt - project officer, work unit #6.27.04A AF25). The compounds reviewed in this report were selected by USARHMA because they were identified as microbial or mammalian metabolites of 2,4,6-trinitrotoluene TNT and 2,4-dinitrotoluene DNT. A knowledge of the potential hazards to man and his environment from munitions manufacturing/loading/disposal etc. has been part of the traditional mission of these two agencies.

The literature review on these compounds of interest was performed at NSWC/White Oak (Code R11) because of our broad chemical knowledge of these compounds, obtained during previous photodegradation/biodegradation/analytical studies on TNT and its major toxic impurity, DNT.

Approved by:

J. F. PROCTOR, Head
Energetic Materials Division

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TABLES

INTRODUCTION

The compounds reviewed in this report were selected by the U. S. Army Toxic and Hazardous Materials Agency. They had been identified as products of microbial or mammalian metabolism of TNT (2,4,6-trinitrotoluene) and DNT (2,4-dinitrotoluene). Molecular formulae, CAS registry numbers, melting points, parent compounds, performance in mutagenicity tests (where done), and appendix designations have been shown in Table 1. Furthermore, CAS registry numbers for certain related compounds, not covered here, have been included. Almost no toxicological information has been found for the compounds in Table 1. There have been no indications that they are more toxic than the parent compounds. The results of literature searches for the first five compounds are to be found in the accompanying appendices. Two azoxy compounds for which searches were requested have evidently not appeared in the literature: 2,4'-dinitro-2',4-azoxytoluene and 2',4-dinitro-2,4'-azoxytoluene.

Although the literature is skimpy, the present overview is valuable, in that it indicated important knowledge gaps as well as the best methods for obtaining the compounds of interest.

TABLE 1. METABOLITES OF TNT AND DNT

Name	Molecular Formula	CAS Registry Number	Melting Point, °C	Parent Compound	Mutagenicity by Ames Assay	App.
4-Hydroxylamino-2,6-dinitrotoluene	$C_7H_7N_3O_5$	59283-75-9	150-151	TNT	Negative[a]	A
4-Acetamido-2-nitrotoluene	$C_9H_{10}N_2O_3$	2719-14-4	147-148	DNT	--	B
2,2′-Dinitro-4,4′-azoxytoluene	$C_{14}H_{12}N_4O_5$	5679-89-0	169-170	DNT	--	C
4,4′-Dinitro-2,2′-azoxytoluene	$C_{14}H_{12}N_4O_5$	67151-57-9	200-201	DNT	--	C
2,2′,6,6′-Tetranitro-4,4′-azoxytoluene	$C_{14}H_{10}N_6O_9$	51857-25-1	215-216	TNT	Negative[a]	D
2′,4,6,6′-Tetranitro-2,4′-azoxytoluene	$C_{14}H_{10}N_6O_9$	51118-04-8	Not available	TNT	--	--
2,4′,6,6′-Tetranitro-2′,4-azoxytoluene	$C_{14}H_{10}N_6O_9$	51856-71-4	Not available	TNT	--	--
4,4′,6,6′-Tetranitro-2,2′-azoxytoluene	$C_{14}H_{10}N_6O_9$	35212-01-2	211-212[b]	TNT	Negative[a]	--

a. Won, W.D., L.H. DiSalvo, and J. Ng. 1976. Toxicity and mutagenicity of 2,4,6-trinitrotoluene and its microbial metabolites. Appl. Env. Microbiol. 31(4):576-580.

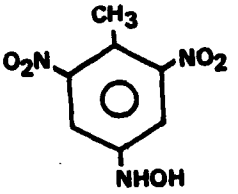
b. Strauss, M.J., S.P.B. Taylor, and A. Resnick. 1972. π and σ interactions of electron-deficient aromatics with amines. Addition to the ring and to a ring substituent. J. Org. Chem. 37(20):3076-3079.

APPENDIX A. CHEMISTRY AND TOXICOLOGY OF 4-HYDROXYLAMINO-2,6-DINITROTOLUENE (4HADNT)

ALTERNATIVE NAMES

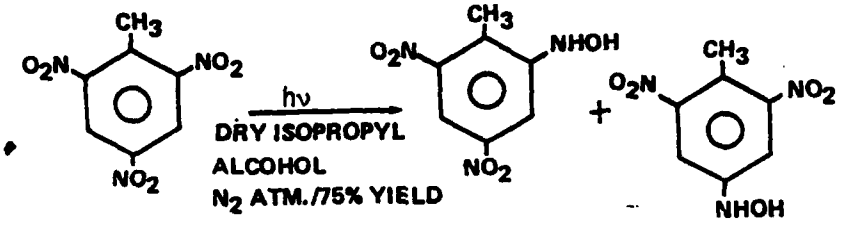
N-Hydroxy-3,5-dinitro-4-methylbenzenamine;
3,5-Dinitro-4-methylhydroxylaminobenzene;
Benzenamine, N-hydroxy-4-methyl-3,5-dinitro (Chem. Abstr. nomenclature, 1976 ff)

PHYSICAL/CHEMICAL PROPERTIES

		Reference
CAS Reg. No.	59283-75-9	
Wiswesser Line Notation:	WNR BI CNW EMO	
Molecular Formula:	$C_7H_7N_3O_5$	
Molecular Weight:	213.15	
Structural Formula:		
Melting point:	143-147°C 150-151°C	1 2
Color:	yellow	1,2
Mass Spectrum (E.I. Mode):	m/e = 213 (M^+)	1
^{1}H-NMR (d_6-acetone):	CH_3 δ 2.39 H_3 & H_5 δ 7.66 NH δ 8.60 OH δ 8.35 J_{NHOH} = 2.0 Hz J_{3Me} = 0.3 Hz	1
TLC (Silica - Merck HF-254):	R_f = 0.25 (benzene)	3
IR (KBr)(cm^{-1}):	3300b, 3275s, 3070, 1525 1330, 1008, 893, 840, 795, 720	4

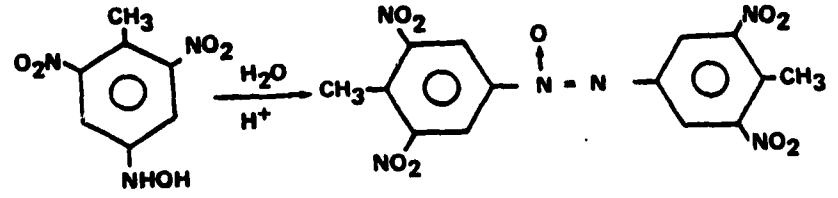
Synthesis

The isolation of pure 4-hydroxylamino-2,4-dinitrotoluene (4HADNT) is difficult, due to contamination by other reduction products. Early claims of synthesis of 4HADNT by the ammonium sulfide reduction of TNT (2,4,6-trinitrotoluene) in ethanol by Elvove,[5] by Anschütz and Zimmermann,[6] and by Ryan and O'Riordan[7] appear, as evidenced by the reported melting points, to have produced 4HADNT contaminated with either the 2-hydroxylamino-4,6-dinitrotoluene (2HADNT) or the correspondingly further reduced 2-amino or 4-amino derivatives. Azoxy formation is also a problem during workup.[8] However, Cohen and Dakin,[9] Lemberg and Callaghan,[10] Burlinson et al.,[4] and Nielsen et al.,[1] apparently have isolated more or less pure samples of 4HADNT. Cohen, Lemberg, and Nielsen used the traditional H_2S/NH_4OH reduction of TNT in ethanol with extensive workup and separation of the reduction products in order to isolate 4HADNT. Lemberg reported the highest mp, 150-151°C (recrystallized from ethanol and washed with benzene) but did not fully characterize the compound. Burlinson, et al.,[4] synthesized 4HADNT by the photolysis of TNT in dry isopropyl alcohol under a nitrogen atmosphere. A 75 percent yield of equal amounts of 4HADNT and 2HADNT was separated by column chromatography (see Annex 1) and characterized by ms and nmr. The spectrometric data agreed with those of Nielsen et al.[1]



Chemical Reactions

When pure, 4HADNT is quite stable in ethanol or benzene solutions, but other TNT reduction products (e.g. 2-amino or 4-amino) tend to catalyze azoxy compound formation.[4,7] Also if acid is present, azoxy formation occurs quite rapidly.[4] Lemberg and Callaghan[2,10] showed that 4HADNT gives the Webster test (brown/purple color) for polynitroaromatics, the Benedict test, and the ammoniacal-silver nitrate test for hydroxylamines. Very little other chemistry is outlined in the literature.



ANALYTICAL METHODS

In the older literature, the most frequently used analytical procedure was colorimetric (Webster test)[11,12] and involved the color generated by the interaction of 4HADNT and KOH in ether. Lemberg and Callaghan[10] used spectrophotometric analysis of TNT reduction products in urine by diazotization and coupling with N-(1-naphthyl)-ethylenediamine. The quantification of 4HADNT was obtained only by difference. These early analytical procedures were subject to much experimental error, especially due to interferences.[2,12]

Gas chromatography of 4HADNT without further derivatization is precluded by its thermal decomposition.[4]

Analysis by nmr seems feasible, based on data of Nielsen et al.,[1] even when the mixture of TNT and its reduction products is present.

Liquid chromatographic methods appear to be the best trace analytic procedures, but none are reported. However, Burlinson has been able to easily separate TNT and some of its reduction products (e.g. 4HADNT, 2-amino-dinitrotoluenes) using reverse phase liquid chromatography with methanol/water (40:60) and 254 nm detection (see Annex).

TOXICOLOGY

Mammalian Metabolism and Metabolites

Bueding and Jolliffe[13] did "in vitro" studies with TNT in various tissue extracts. 4HADNT appeared as an intermediate metabolic product with 4-aminodinitrotoluene as the end product (Webster Test). They found that TNT is reduced by partially purified xanthine oxidase to 4HADNT. They suggest a stepwise reduction of

TNT via nitro ⟶ nitroso ⟶ hydroxylamino ⟶ amino.

Lemberg and Callaghan[10] found 4HADNT in human urine (volunteers fed TNT and munition workers) by the Webster Test. Channon et al.,[14] found 4HADNT in the urine of rabbits fed TNT. Lemberg and Callaghan[2,10] also found 4HADNT in the urine of rats fed TNT.

Haas[15] found that 4HADNT, when dissolved in olive oil and shaken with blood or a suspension of washed corpuscles, rapidly converted hemoglobin to methemoglobin.

Microbial Metabolism and Metabolites

In a microbial metabolite study by Won, Heckley, Hoffsommer, and Glover, laboratory-cultured pseudomonas isolates were shown to produce 4HADNT from TNT along with the other amino and azoxy reduction products.[16]

Later, Won, DiSalvo and Ng reported that Ames tests showed TNT to be a frameshift mutagen (TA-98), but in contrast, the major microbial metabolites of TNT, including 4HADNT, appeared to be nontoxic and nonmutagenic.[17]

Recent Russian work by Naumova et al.,[18] postulated 4HADNT and 2HADNT as intermediates in the microbial reduction of TNT with Pseudomonas denitrificans.

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ANNEX 1 TO APPENDIX A

SYNTHESIS AND ANALYSIS OF 4-HYDROXYLAMINO-2,6-DINITROTOLUENE (4HADNT)

by N. E. Burlinson

Synthesis (Photochemical Method)

Three grams of TNT is dissolved in 600 mL of dry isopropyl alcohol (distilled from sodium) and placed in a 1 L Ace photoreactor containing a pyrex cooling well with a 500-W medium pressure mercury lamp. Under a N_2 atmosphere, the solution is irradiated for 6 hours. The resulting yellow solution is taken to dryness with 20 g of silica on a rotary evaporator (40°C). The remaining solid is chromatographed on 200 g of silica gel, at first with 500 mL of benzene to remove unreacted TNT, then with about 500 mL of 3 percent ethyl acetate/benzene. A 75 percent yield of equal amounts of 4HADNT and 2HADNT is obtained (with the 4HADNT emerging first).

Note: This method may be more time-consuming than that of Elvove[1] (NH_4OH/H_2S in EtOH) but it is reported here since it is the best method for 2HADNT synthesis.

LC Analysis

Water Model 240 High-Performance Liquid Liquid Chromatograph with RCSS unit
Detector - Waters 440 UV (254 nm)
Column - Waters Radial PAK B (C^{18}) reverse phase
Solvent - 40:60 methanol/water
Retention Time - 12 min at 2 mL/min-slow rate

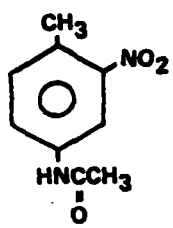
1. Elvove, E. 1919. The detection and estimation of small amounts of certain organic compounds with special reference to the examination of the urine of TNT workers. J. Ind. Eng. Chem. 11:860-864.

APPENDIX B. CHEMISTRY OF 4-ACETAMIDO-2-NITROTOLUENE (4Ac2NT)

ALTERNATIVE NAMES

4-Acetamido-2-nitromethylbenzene;
2-Nitro-p-acetotoluidine;
2-Nitro-4-acetamidotoluene;
Acetamide, N-(4-methyl-3-nitrophenyl) (Chem. Abstr. nomenclature)

PHYSICAL/CHEMICAL PROPERTIES

		Reference
CAS Reg. No.:	2719-14-4	
Wiswesser Line Notation:	WNR BI EMVI	
Molecular Formula:	$C_9H_{10}N_2O_3$	
Molecular Weight:	194	
Structural Formula:		
Melting Point:	145-146°C	1
	148.5°C	2
	147-148°C	3
UV Absorption Spectrum:	max = 365 nm (cyclohexane)	4
NMR ($CDCl_3$):	NH (excessively broadened resonance) CH_3 2.50 δ $COCH_3$ 2.15 δ H_3 7.88 δ H_5 7.55 δ H_6 7.08 δ	5
Mass Spectrum:	m/e = 194(M^+), 135, 107	6

Synthesis

Starting with 2,4,6-trinitrotoluene (TNT), Niemann, et al.[1] reduced the 4-nitro group by the method of Elvove,[7] using ammonium hydroxide/H_2S, to obtain 4-amino-2,6-dinitrotoluene, which was then acetylated with acetic

anhydride. One of the two nitro groups was removed by treatment of the dinitroacetamidotoluene with hypophosphorous acid and sodium nitrite[8] to give a 50 percent yield of 4Ac2NT after recrystallization from ethanol (mp, 145-146°C).

Starting with 2,4-dinitrotoluene, Jadot[3] et al. were able to reduce the 4-nitro group with Raney copper and hydrogen gas in benzene to obtain 4-amino-2-nitrotoluene, which was then easily acetylated with acetic anhydride to 4Ac2NT (mp, 147-8°C).

Another similar route by Ferguson[9] was to nitrate p-toluidine with nitric acid/sulfuric acid at -15°C, followed by acetylation of the nitrated product to 4Ac2NT (mp 142-3°C).

The easiest method for preparation of 4Ac2NT is that of McCormick[6] who acetylated commercially available 4-amino-2-nitrotoluene by refluxing 30 min with 3 mL of acetic anhydride. The compound, recrystallized from ethanol/water, melted at 144°C.

Reactions

Very few reactions of 4-acetamido-2-nitrotoluene are mentioned in the literature. The nitration of 4Ac2NT by Dey et al.[10] gave 2,5-dinitro-4-acetamidotoluene (25 percent yield), which could then be hydrolyzed with acid to 2,5-dinitro-4-aminotoluene.

ANALYTICAL METHODS

Gas chromatography of 4Ac2NT on nine different columns was documented in the literature by Ono,[11] who was able to separate it from other acetamidotoluene isomers. He used several liquid phases on 60-80 mesh supports. Two of those phases gave the following retention times for 4Ac2NT.

Gas Chromatograph - Shimadzu Model GC-5A
Column - 10% Ethylene Glycol Phthalate on Sil-O-Cel C_{22} Firebrick (Johns Manville) in a 0.75 M x 3 mm ID Stainless Steel V-Tube
Hydrogen Flow Rate - 20 mL/min
Detector - FID @ 220°C
Retention Time = 38.80 min for 4Ac2NT

and

Column - 3% OV -17 on Celite 545 in a 1.5 M x 3 mm ID Stainless Steel V-Tube
Other Conditions as Above
Retention Time = 8.30 min for 4Ac2NT

Thin-layer chromatography (tlc) has been reported by Ono as well.[12] He used silica gel GF_{254} (type 60) from E. Merck, which was coated to 250 μm thickness and activated for 1 hour at 110°C. Solvents for separating 4Ac2NT from other isomers were (A) chloroform-ethyl acetate (9:1); (B) carbon tetrachloride-ethanol (85:10:5) and (C) cyclohexane-chloroform-ethyl acetate (50:20:20).

R_f in (A) = 0.35; R_f in (B) = 0.20; R_f in (C) = 0.12. (Not separated from 3Ac6NT by any solvent, poorly separated from 3Ac5NT.)

Although liquid chromatographic analysis of 4Ac2NT is not reported, these authors feel that for dilute solutions reverse phase - C_{18} column could easily be used with methanol/water or acetonitrile/water as solvent and UV detection (λ = 254).

METABOLISM

McCormick, et al.,[6] reported the presence of 4Ac2NT along with 4-amino-2-nitrotoluene, 2-amino-4-nitrotoluene, 2,2'-dinitro-4,4'-azoxytoluene, and 4,4'-dinitro-2,2'-azoxytoluene, plus another unidentified mixed azoxy compound from the microbial transformation of 2,4-dinitrotoluene by Microsporium sp. The transformation products were isolated by tlc on 250 µm thick silica gel (Kontes/Quantum Preadsorbent TLC-LODF) by development in benzene/hexane (1:1). Spots were visualized by UV, scraped off the plate and extracted with dichloromethane. The concentrated extracts were then analyzed by GC/MS using glass columns packed with 3 percent OV-17 on Gas-Chrom Q.

Bond and Rickert[13] reported 4Ac2NT as a minor metabolic product of the hepatic metabolism of 2,4-dinitrotoluene; the major end product was 2,4-dinitrobenzyl alcohol glucuronide.

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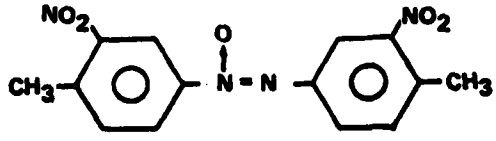
APPENDIX C. CHEMISTRY OF AZOXYTOLUENE ISOMERS DERIVED FROM 2,4-DINITROTOLUENE

1. 2,2'-DINITRO-4,4'-AZOXYTOLUENE

ALTERNATIVE NAMES

3,3'-Dinitro-4,4'-dimethylazoxybenzene;
Diazene, bis(4-methyl-3-nitrophenyl)-1-oxide (Chem. Abstr. nomenclature)

PHYSICAL/CHEMICAL PROPERTIES

		Reference
CAS Reg. No:	5679-89-0	
Wiswesser Line Notation:	WNR B1 ENUNO & D1 CNW	
Molecular Formula:	$C_{14}H_{12}N_4O_5$	
Molecular Weight:	316.081	
Structural Formula:		
Mass Spectrum:	m/e = 316(M^+), 106, 104	1
Melting Point:	169-170°C	

Synthesis

The earliest mention of 2,4-dinitro-4,4'-azoxytoluene was by Brand and Löller.[2] They isolated it during the electrochemical reduction of 2,4-dinitrotoluene (DNT), claiming to obtain it as slender, pale, yellow needles (mp, 164°C). In addition, they isolated 4-hydroxylamino-2-nitrotoluene (mp, 99°C) and 4-nitroso-2-nitrotoluene (mp, 87°C). In 1941, Albert and Ritchie[3] reported the isolation of 2,2'-dinitro-4,4'-azoxytoluene from the hydrogen/Raney nickel reduction of 2,4-dinitrobenzaldehyde. In another reduction of DNT, Chen and Wu[4] used n-butylmercaptan and potassium hydroxide in ethanol to obtain 62 percent crude yields of 2,2'-dinitro-4,4'-azoxytoluene. To purify, they recrystallized it from 95 percent ethanol, then chromatographed it on alumina with benzene (mp, 164.5°C).

The most recent synthesis, and probably the simplest for 2,2'-dinitro-4,4'-azoxytoluene was that of McCormick, Cornell, and Kaplan.[1] They treated 4-amino-2-nitrotoluene in dichloromethane with two molar equivalents of m-chloroperbenzoic acid. After the reaction mixture had stood overnight at ambient temperature, the precipitate of m-chlorobenzoic acid was filtered off, the dichoromethane filtrate extracted with 5 percent sodium bicarbonate, and the organic layer evaporated. The solid product was recrystallized from 95 percent ethanol to give a slightly yellow solid (mp, 169-170°C).

ANALYTICAL METHODS

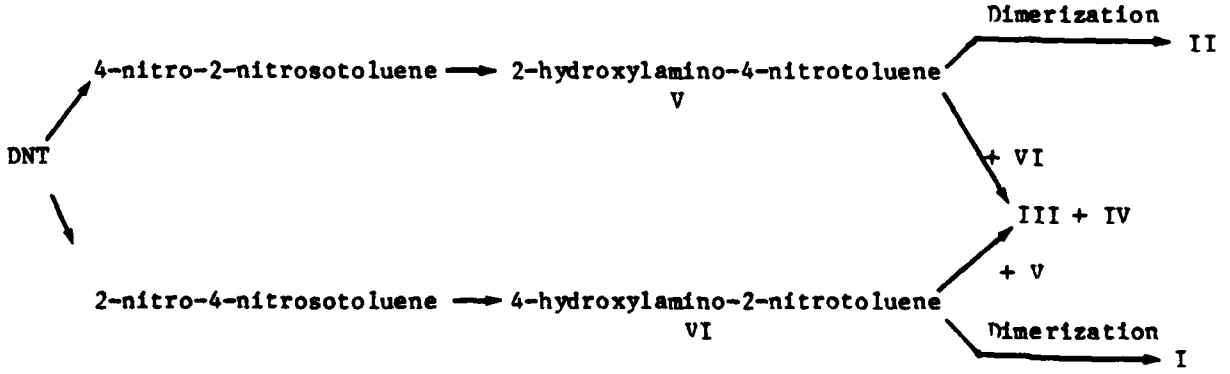
Thin-layer chromatography[1] was used to separate 2,2′-dinitro-4,4′-azoxytoluene from the azoxy isomers and from other microbial reduction products of DNT. The conditions for tlc were: Kontes/Quantum Preadsorbants silica gel plates TLC-LQDF, 250 μm thickness, developed with benzene-hexane (50:50). The tlc bands could be detected with UV light.

Gas chromatography/mass spectrometry (GC/MS) was also reported;[1] the glass column was packed with 3 percent OV-17 on Gas-Chrom Q. The mass spectrum showed peaks at m/e 316(M^+), 106 and 104.

No other analytical data have been reported for 2,2′-dinitro-4,4′-azoxytoluene.

METABOLISM

McCormick et al.,[1] found 2,2′-dinitro-4,4′-azoxytoluene (I) as a microbial transformation product of the action of Microsporium sp. on DNT. This compound was isolated and identified by thin-layer chromatograhy and gas chromatography/mass spectrometry, respectively, along with the other transformation products, 2-amino-4-nitro-toluene, 4-amino-2-nitrotoluene, 4,4′-dinitro-2,2′-azoxytoluene (II), 4-acetamido-2-nitrotoluene and a third azoxy compound believed to be a "mixed" azoxy not yet identified, i.e., 2,4′-dinitro-2′,4-azoxytoluene (III) and 2′,4-dinitro-2,4′-azoxytoluene (IV). They suggested that the azoxy compounds are formed by non-enzymatic pathways (i.e., by oxidative coupling of hydroxylamino compounds). 2,4-Dinitrotoluene, at 100 mg/L, was introduced to Microsporium sp. and incubated on a synthetic medium containing glucose. The proposed pathways for transformation to the azoxy isomers appear as follows:[1]

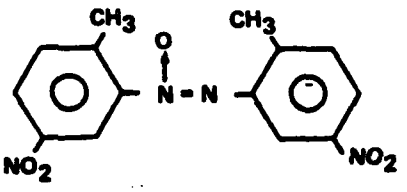


2. 4,4'-DINITRO-2,2'-AZOXYTOLUENE

ALTERNATIVE NAMES

2,2'-Dimethyl-5,5'-dinitroazoxybenzene;
Diazene, bis(2-methyl-5-nitrophenyl)-1-oxide (Chem. Abstr. nomenclature)

PHYSICAL/CHEMICAL PROPERTIES

		Reference
CAS Reg. No.	67151-57-9	
Wiswesser Line Notation:	WNR D1 CNUNO & B1 ENW	
Molecular Formula:	$C_{14}H_{12}N_4O_5$	
Molecular Weight:	316.081	
Structural Formula:		
Melting Point:	200-201°C	1
Mass Spectrum:	m/e = 316(M^+), 301, 90	1
Thin-Layer Chromatography:	Silica gel/benzene:hexane (1:1 by vol.). Visualization-UV R_f = 0.5	1

Synthesis[1]

Only one reference was found, namely that of McCormick et al.[1] They used a modified method of Sitzmann[5] to synthesize 4,4'-dinitro-2,2'-azoxytoluene from 2-amino-4-nitrotoluene and m-chloroperbenzoic acid in dichloromethane.

ANALYTICAL METHODS[1]

The methods for 4,4'-dinitro-2,2'-azoxytoluene are essentially the same as for 2,2'-dinitro-4,4'-azoxytoluene, with slight differences in tlc and gc/ms. The tlc spot of the former comes just below that of the latter and the mass spectral data for the two include the same molecular ion, but different species by fragmentation.

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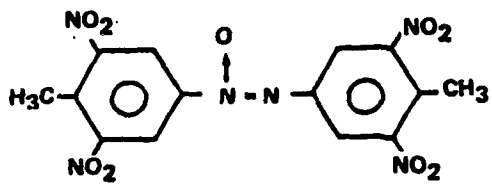
APPENDIX D. CHEMISTRY AND TOXICOLOGY OF 2,2',6,6'-TETRANITRO-4,4'-AZOXYTOLUENE

ALTERNATIVE NAMES:

3,3',5,5'-Tetranitro-4,4'-dimethylazoxybenzene;
2,6-Dinitro-4-azoxytoluene;
Diazene, bis(3,5-dinitrophenyl-4-methyl)-1-oxide (Chem. Abstr. nomenclature)

		Reference
CAS Registry Number:	51857-25-1	
Wiswesser Line Notation:	WNR B1 CNW ENUNO & RD1 CNW ENW	
Molecular Formula:	$C_{14}H_{10}N_6O_9$	
Molecular Weight:	406	
Structural Formula:		
Melting Point:	215-16°C	1,2
Color:	white	1,2
Mass Spectrum (EI mode):	m/e = 406(M^+)	3
^{1}H-NMR (d_6-DMSO):	δ2.55s CH_3 δ2.52s CH_3 δ9.08s aromatic δ8.91s aromatic	2
tlc (silica-G-60):	R_f = 0.8 (benzene); visualization with UV (254) or spray Reagent-ethylenediamine/DMSO (2:10 v/v); color is blue	8,9

Synthesis

The early work of Brand and Eisenmenger[1] and also of Anschütz and Zimmermann[4] demonstrated the formation of 2,2',6,6'-tetranitro-4,4'-azoxytoluene by the acid-catalyzed condensation of 4-hydroxylamino-2,6-dinitrotoluene (4HADNT).

Several attempts to isolate the reduction products of TNT (e.g. 2-amino, 4-amino, or the 2 or 4 hydroxyamino-dinitrotoluenes) resulted in formation of the alcohol-insoluble azoxy compounds, according to the literature.

Solutions of 4HADNT in water or alcohol give rise, on standing, to 4-azoxy- and 4-amino-2,6-dinitrotoluene.[5] It appears that the presence of oxygen, peroxides, acid, or amino compounds catalyzes azoxy formation following the hydroxylamine reduction stage. It is well known that nitroso and hydroxylamino functions readily couple to form the azoxy linkage.[6,7]

Sitzmann found one of the more facile methods for 4-azoxy formation to be treatment of 4-amino-2,6-dinitrotoluene with m-chloroperbenzoic acid in methylene chloride.[2] The general principle, as outlined in P.A.S. Smith's book, "The Chemistry of Open-Chain Organic Nitrogen Compounds"[6] is illustrated below.

$$RNH_2 \xrightarrow{[o]} RNHOH \xrightarrow{[o]} RNO + H_2O$$

$$RNHOH + RNO \longrightarrow RN(OH)-N(OH)R \longrightarrow R-\overset{\overset{O}{\uparrow}}{N}=N-R + H_2O$$

$$RNH_2 + RNO \longrightarrow RNHN(OH)R$$

$$RN=NR \xrightarrow{[o]} RN=\overset{\overset{O}{\uparrow}}{N}R$$

Chemical Reactions

No specific chemical reactions are reported in the literature for this 4-azoxy derivative of TNT. It is generally reported as one of the end products from TNT reduction. Because it is extremely insoluble in water, further reduction of its four nitro groups had not been reported. This author has observed that in solvents such as tetrahydrofuran and isopropyl alcohol, further photochemical reductions, followed by azo or azoxy coupling, can occur to form higher ring homologues.[8]

ANALYTICAL PROCEDURES

The mass spectrum of 4-azoxy was studied by Kubose and Glover,[3] who found a Varian Mat-111 low-resolution mass spectrometer (70ev ionization potential) to give a suitable spectrum. A parent, ion at m/e = 406 was observed, with the most prominent peaks at M-17(OH) and m/e = 209, 181, 134, and 89.

Thin-layer chromatography (tlc) has been employed both by Burlinson et al.,[8] and by Won, et al.,[9] for separation of azoxy compounds derived from TNT. By use of Polygram Sil G (Brinkmann) in the ascending method, with a solvent system comprised of toluene/benzene/hexane (10:10:5 by volume), a mixture of the 4-azoxy and 6-azoxy compounds could be separated easily. ($R_f \cong 0.8$ and 0.6 respectively).[9] The spray reagent, ethylenediamine/dimethylsulfoxide (2:10 by vol.) can be used for visualization. Tetranitro-azoxy isomers turn blue or purple when sprayed.[8]

Gas chromatography of tetranitro-azoxy compounds as a group is impossible owing to thermal decomposition at the necessary high column temperature.

No liquid chromatographic (LC) methods have been reported but LC methods would appear most suitable. Reverse phase LC could be used if the concentration were below 10 mg/L.

Nmr analysis of the compound in dimethyl sulfoxide was reported by Sitzmann;[2] the data are given in the "Physical/Chemical Properties" section of this report.

TOXICOLOGY

Mammalian Metabolism

Dale, et al. (1921)[10] reported isolating 2,2′,6,6′-tetranitro-4,4′-azoxytoluene from the urine of rabbits after subcutaneous injections of TNT. However, Lemberg and Callaghan,[11] and Channon, Mills and Williams[12] showed, after repeating Dale's work, that 2,2′-6,6′-tetranitro-4,4′-azoxytoluene was not a metabolite, but formed during workup of 4-hydroxylamino-2,6-dinitrotoluene, which was a metabolite. Snyder,[13] also did not observe this compound as a metabolite in the urine of dogs fed TNT.

Bueding and Jolliffe[5] did in vitro studies with TNT, using liver extracts, pig heart enzymes, and xanthine oxidase. Only the hydroxylamino and amino reduction products were observed. 2,2′,6,6′-Tetranitro-4,4′-azoxytoluene was only seen to form during the workup of the 4HADNT metabolite in ether.

Microbial Metabolism

Microbial metabolism of TNT has been shown to produce 2,2′,6,6′-tetranitro-4,4′-azoxytoluene. Won et al.[9] observed the formation of this product along with the other isomers and reduction products of TNT from pseudomonad isolates (pure strains) under aerobic conditions. However, it is likely not a direct product but a substance formed from the coupling reactions of the corresponding hydroxylamines. The 4-azoxy, 6-azoxy, 2-amino and 4-amino-reduction products of TNT were also observed by Naumova et al.,[14] during the first stage of TNT transformation by Pseudomonas denitrificans.

It is interesting to note, however, that when TNT was biotransformed under aerobic mixed culture conditions (i.e., activated sludge microorganisms and supplemental nutrient) in a biooxidation ditch, Hoffsommer et al.[15] were never able to detect 2,2′,6,6′-tetranitro-4,4′-azoxytoluene or any other azoxy isomers building up over a 3-year period.

Toxicity and Mutagenicity

Won, DiSalvo, and Ny[16] studied 2,2′,6,6′-tetranitro-4,4′-azoxytoluene under conditions where TNT was toxic and mutagenic. They found it and other TNT reduction products to be nontoxic to algae, copepods and oyster larvae, and nonmutagenic to Salmonella typhimurium (Ames test).

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